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ACUTE T-2 INTOXICATION: PHYSIOLOGIC CONSEQUENCES AND NEW THERAPEUTIC APPROACHES

FINAL REPORT

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FINAL REPORT

Contract Effort: 86PP6807

Title: Acute T-2 intoxication: physiologic consequences and new therapeutic approaches

Objective: The objectives of this contracts were two: 1) to study the physiologic consequences of acute intoxication with T-2 mycotoxin; 2) to develop therapeutic modalities to reduce morbidity and mortality from severe T-2 toxin exposure.

SPECIFIC AIMS

Specific Aim 1

To characterize the cardiorespiratory hematological and general autonomic responses to mild, moderate or severe exposure to T-2 mycotoxin.

These studies were conducted in rats and guinea pigs which were chronically instrumented to allow direct monitoring of cardiorespiratory variables. Some studies were also conducted in pithed rats to evaluate peripheral effects of T-2 toxin without modulatory effects of the central nervous system (baroreflexes, etc.). All studies were conducted in a dose-response manner. In the rat, T-2 toxin at (0.5-2.0 mg/kg, i.v.) produced hyperdynamic state marked by hypertension and tachycardia followed by hypotension and shock. Marked increases in total peripheral resistance underlied the hypertensive phase as cardiac output was reduced. In guinea pigs, T-2 toxin (0.5-2.0 mg/kg, i.v.) only hypotension and bradycardia were observed. All doses of T-2 toxin produced mortality; in rats, 0.5, 1.0 or 2.0 mg/kg resulted in 61, 24 and 0% survival, respectively,. While, in guinea pigs, the same dosing regimen resulted in 72 57 and 20% survival. Interestingly, in pithed rats, T-2 toxin failed to elicit the early hypertensive/tachycardic response, indicating a central mediated mechanism of this hyperdynamic state (these observations led to the studies on the central effects of T-2, vide supra). In rats, marked stimulation of the sympatho-adrenomedullary system was evidenced by elevated circulating levels of norepinephrine, epinephrine and dopamine.

Furthermore, significant neuroendocrine derangements were noted by elevated circulating levels of vasopressin and selected prostanoids (e.g., prostacyclin but not LTC_4 or TXA_2).

The most prominent consequences to T-2 toxin administration in both guinea pigs and rats were severe acidosis, hypocarbia, without hypoxemia. In fact, hypoxemia was dose-dependent in both species. These metabolic changes were also observed in pithed rats suggesting peripheral organ toxication of aerobic metabolism. Additional interesting features of T-2 toxemia were: enhanced epinephrine release from spinal cord stimulated pithed rat and modest elevation of hematocrit. Taken together, these comprehensive studies in two species clearly indicated high toxicity of T-2 toxin and a broad, multi-organ/multisystem involvement. These studies were described in detail in *The Journal of Pharmacology and Experimental Therapeutics* 232:786-794, 1985.

Specific Aim 2

To characterize in more detail the hemodynamic derangements monitored in the first study (vide supra), rats were chronically instrumented with Doppler flow probes which allow to follow selected organ blood flow and vascular resistance In conscious rats, intravenous administration of T-2 toxin (1 mg/kg), resulted in reduced blood flow and increased vascular resistance in hindquarters, mesenteric and renal vascular beds. The reduction in hindquarter blood flow preceded the renal and mesenteric flows but, ultimately (30-60 min post-dosing), blood flows to all organs were severely compromised. Changes in systemic blood pressure was moderate, presumably due to the counteracting effect of the reduced cardiac output.

Since this study revealed renal hypoperfusion, a phenomenon known to result in activation of the renin-angiotensin system, the circulating levels of renin (as PRA) were monitored. Indeed, T-2 toxin (0.75 mg/kg, i.v.) resulted in marked increase in PRA, at the time of severe renal hypoperfusion only (>1 hr post T-2 dosing). Taken together, this study confirmed the previous report where marked increase in total peripheral resistance was noted and further extended this observation to selected somatic and splanchnic organs. The study also reported the activation of the third pressor system, renin-angiotensin, in addition to activation of the sympatho-adrenomedullary system and vasopressin release. Most importantly, the severe reduction in organ blood flow explained the marked metabolic (lactic) acidosis, typical to organ ischemia.

These experiments are described in detail in the publication *Toxicology and Applied Pharmacology* 83:438-444, 1986.

Specific Aim 3

Evidence in support for central effects of T-2 toxin has been generated by both our own work (vide supra) as well as other reports published at the time of conducting our own research. First, we have followed the vasopressin response (marked release) and explored its content in the pituitary gland along with oxytoxin, a peptide co-stored and released with vasopressin. Indeed, T-2 toxin (1 mg/kg, i.v.) depleted much of the vasopressin and oxytoxin in the posterior pituitary gland; interestingly, the opioid peptide, leu-enkephalin, was also reduced while methionine-enkephaline content increased. Dynorphin content did not change. Thus, T-2 toxemia seems to produce a highly differentiated neuroendocrine response These data were published in detail (*Science* 228:606-608, 1985).

Furthermore, we followed the eicosanoid lead described in Specific Aim 1, i.e., selective increase in plasma prostacyclin in T-2 toxemia. To this end, brain slices were studied *in vitro* (rat) following T-2 toxin dosing (0.75-2.0 mg/kg, i.v.) or *in vitro* exposure of brain slices to a wide range of T-2 toxin concentrations. *Ex vivo* assay of the eicosanoids in brain slices of *in vivo* dosing with T-2 toxin enhanced release of 6-keto-$PGF_{1\alpha}$ (stable metabolite of PGI_2) and TXB_2 (stable metabolite of TXA_2) in cortical but not in the hypothalamus or nucleus tactus solitarium (NTS). PGE_2 showed time and dose related increments in both the cortex and hypothalamus but not NTS. *In vitro* incubation of cortical slices with T-2 toxin (10^{-9}-10^{-3}M) demonstrated a complex response: stimulation of PGE_2 and TXB_2 release from cortical slices at low (10^{-7}M) concentration and inhibition at high (10^{-4}M). These data elude to a direct effect of T-2 toxin on tissue phospholipase A_2 (activation) but also inhibitory effects on special eicosanoid metabolic pathways. These studies were published in detail in *Prostaglandins* 31:307-319, 1986.

In addition to neurochemical effects of T-2 toxemia, we have also studied behavioral and autonomic responses to T-2 toxin. Direct administration of T-2 toxin into the cerebroventricular (ICV) system of the rat produced cardiovascular responses at doses which had no such effect when given systemically. This, ICV T-2 toxin at 100μg/kg, elicited pressor and tachycardic responses; a higher dose, 300 μg/kg, ICV, produced progressive and sustained hypertension and tachycardia compared to only transient effects upon systemic administration. Furthermore, at 300 mg/kg, ICV, only 50% of the rats survived at 24 hr, while the same dose given systemically did not result in any mortality by 72 hr post-dosing. These studies were

published in *Trichothecene Mycotoxins* Vol. 11:111-122, 1989, V. R. Beasley, ed., CRC Press.

Specific Aim 4

A major goal of our research on T-2 toxemia was the development of therapeutic approaches to this severe toxic agent.

A. Studies with PAF antagonist, BN52021

Our first approach to combat T-2 toxicity stemmed from studies with platelet-activating factor (PAF) antagonists in lipopolysaccharide (LPS) endotoxemia where various PAF antagonists have shown significant protection and reduced mortality from LPS-lethal toxemia. We, therefore, utilized a specific PAF-antagonist, BN52021, in our conscious rat model where an ED_{50} dose of T-2 was given i.v. and the PAF antagonist administered at various time points before and/or post T-2 dosing. In three separate experiments, BN52021 significantly enhanced survival (3 day, final). The most significant study was that where BN52021 (15 mg/kg) given 3 hrs post T-2 dosing significantly (more than tripled) enhanced survival rate. BN52021 is now in Phase III clinical development for treatment of endotoxemia (gram negative) and is potentially of great value also for T-2 toxemia. This study was published in *Toxicology Letters* 38:271-274, 1987.

B. Studies with Dexamethasone

Glucocorticosteroids are known to protect animals against lethal doses of endotoxins, especially wnen given as prophylactic therapy. Glucocorticosteroids are also known to suppress the formation of eicosanoids, which were argued to play a role in the pathomechanisms of a variety of toxemias. Since we have found evidence in support of activation of eicosanoid synthesis also in T-2 toxemia, we have chosen to test the efficacy of dexamethasone, a potent, synthetic glucocorticosteroid in our rat T-2 toxemia model. Dexamethasone (2 mg/kg, i.v.) was given 1 hr post T-2 dosing; at 10 mg/kg, 1 and 24 hr post T-2 dosing; 1 mg/kg at 1, 24 and 48 hr post dosing or 10 mg/kg at 3 hr post T-2 toxin. The triple dosing (1 mg/kg, 1, 24 and 48 hr) resulted in remarkable increased survival (over 60% compared to 0% in controls); the other protocols also improved survival at various (but lesser) degrees. Interestingly, the dual cycloxygenase/lipoxygenase inhibitor BW755c, failed to provide any consistent protection against

T-2 toxemia Dexamethasone also protected against T-2 toxin induced inhibition of PGE_2 synthesis in brain 24 hr post dosing.

This study was published in *Pharmaceutical Research* 4:527-530, 1987.

C. Studies with monoclonal antibodies against T-2 toxin

In collaboration with Dr. K. Hunter, a monoclonal antibody (MAb) against T-2 toxin has been raised (mouse IgG1) and labeled 15H6. These MAbs were shown to induce net efflux of ^{3}H-T-2 toxin from poisoned human B-lymphoblastoid cells in vitro and restore protein synthesis. Administration of the MAb (250 mg/kg, i.v.) 30 min before infusion of lethal dose of endotoxin (1 mg/kg, i.v.) caused sequestration of the toxin in the plasma compartment. Administration of the MAb 35 min after the T-2 administration resulted in migration of T-2 toxin back into the plasma. These data (published in *The Journal of Pharmacology and Experimental Therapeutics* 255:1183-1187, 1990) demonstrate that monoclonal antibodies can be of therapeutic value against an intracellular toxin and, specifically, T-2 toxin.

The above studies prompted us to investigate the therapeutic capacity of 15H6 MAb in lethal T-2 toxemia. In our conscious rat T-2 toxemia model, where 1 mg/kg T-2 caused 100% mortality, 15H6 treated rats (250 mg/kg, i.v., 30 min after or 15 min after T-2 dosing) demonstrated 100% protection. When 15H6 was given 60 min post, T-2 still produced 45% protection.

Taken together, our studies with 15H6 MAb have resulted in a pioneering experience of a novel strategy to salvage cells/organs from actions of intracellular toxins and, in particular, successful use of this antibody as a tool for prophylactic and therapeutic utility (Study published in *J Clin Invest* 76:2134-2138, 1985).

SUMMARY

Our research on T-2 toxemia has resulted in comprehensive evaluation of all major systems and, in particular, the cardiovascular, respiratory, autonomic and neuroendocrine. Our research has also led to identification of three different therapeutic modalities: PAF antagonists, dexamethasone and monoclonal antibodies (15H6). The latter strategy could well be further developed into human use by "humanizing" the 15H6 antibody through molecular engineering approaches. This research program was exciting, scientifically rewarding and of significant clinical potential.

On a final note, I maintain deep respect and gratitude to USMRDC/USAMRIID for sponsoring such research which clearly benefits both the military and civilian society.

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